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Inhibition of Morphine-Induced Analgesia and Locomotor Activity in Strains of Mice: A Comparison of Long-Acting Opiate Antagonists

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FRISCHKNECHT, H.-R., B. SIEGFRIED, G. RIGGIO AND P. G. WASER. *Inhibition of morphine-induced analgesia and locomotor activity in strains of mice: A comparison of long-acting opiate antagonists.* PHARMACOL BIOCHEM BEHAV **19**(6) 939–944, 1983.—The long-acting opiate antagonistic potency of naloxazone (NXZ), β-chlornaltrexamine (β-CNA) and β-funaltrexamine (β-FNA) was compared using three inbred strains of mice, in which morphine induces either analgesia (DBA/2), locomotion (C57BL/6), or both responses (C3H/He). The antagonists were applied SC 24–120 hr before morphine (10 or 20 mg/kg, IP), followed by the tests after 30 min. The minimal dose which completely antagonized morphine-induced analgesia in DBA and locomotion in C57 mice during 24 hr were: for NXZ 50 and 100 mg/kg, for β-CNA 0.8 and 6.2 mg/kg, for β-FNA 1.6 and 12.5 mg/kg, respectively. β-FNA and β-CNA more potently blocked morphine-induced analgesia in DBA mice than the activity response in the C57 strain. In contrast, β-FNA prevented morphine-induced locomotion at a lower dose (6.2 mg/kg) than analgesia (>50 mg/kg) in C3H mice, while β-CNA was equipotent (1.6 mg/kg). In general, β-CNA turned out to be the most reactive compound, antagonizing morphine effects in low doses up to 120 hr. β-FNA selectively antagonized either morphine-induced analgesia or locomotion, depending on the strain used. This suggests that a given morphine response might be caused by a genetically determined multiplicity of opiate receptor types and their mutual interactions.

Long-acting opiate antagonists Naloxazone β-Chlornaltrexamine β-Funaltrexamine
Mice Inbred strains C57BL/6 DBA/2 C3H/He Morphine Analgesia Locomotion

DURING the last few years an unequivocal need for long-acting substances with pure opiate antagonist properties became evident. Their possible therapeutic application in the treatment of opiate addiction and readdiction, as well as of obesity was discussed [6]. Furthermore, irreversibly binding opioids would be useful pharmacological tools in characterizing and isolating opiate receptors.

Several long-acting, irreversible opiate antagonists were described recently: β-chlornaltrexamine [17], β-funaltrexamine [18], naloxazone and naltrexazone [15]. Long-term potency of these substances was demonstrated mainly by in vitro experiments on guinea pig ileum, mouse vas deferens and brain homogenates, as well as by in vivo measurement of analgesia [14, 16, 24]. By using different agonists, which preferentially act on μ-, δ- or κ-receptors in the above mentioned preparations, a selectivity to block μ-mediated responses was shown for β-funaltrexamine in contrast to β-chlornaltrexamine [22, 24, 25]. Similarly, naloxazone was reported to selectively block analgesia mediating high affinity (μ_1) binding sites in contrast to low affinity (μ_2 and δ) binding sites [13,26].

Inbred strains of mice represent a useful tool for the study of opiate action, in that marked strain differences in the response to morphine action in vivo are evident [1, 4, 20]. For instance, in C57BL/6 mice the preferential response to morphine is an increase in activity, while DBA/2 mice show a pronounced analgesic effect [12]. Differences in the ratios of μ- and δ-receptors have been found in these strains, i.e., in comparison to DBA mice, C57 mice show higher number of striatal δ-receptors, while no differences were found for μ-receptors in the striatum and other brain structures [19]. The preponderance of one opiate receptor subtype may be of importance for the expression of a preferential response to morphine. It is suggested, that the analgesic effects are mediated mainly by μ-receptors, whereas behavioral effects are mediated mainly by δ-receptors [2, 5, 10, 11, 21]. Based on this assumption, we hypothesized that a selective μ-antagonist should block more selectively the analgesia response in DBA/2 mice in comparison to the running fit in C57BL/6 mice. Thus the present work compared the three long-term antagonists β-chlornaltrexamine, β-funaltrexamine and naloxazone in two in vivo models of morphine action, and tested the above mentioned hypothesis.

EXPERIMENT 1

The aim of Experiment 1 was to assess the antagonistic potency of β-chlornaltrexamine, β-funaltrexamine and

naloxazone on morphine-induced analgesia in DBA/2 mice as well as on morphine-induced locomotor activity in C57BL/6 mice.

METHOD

Subjects

Male mice of the C57BL/6 (C57) and the DBA/2 (DBA) strain (Institut für Zuchthygiene, Universität Zürich) were used. Mice of both strains were housed (Macrolon cages 42×26×17 cm) in groups of ten animals upon arrival and tested 2–3 weeks later at an age of 8–10 weeks (24–26 g). They were kept under a natural light/dark cycle with food and water ad lib.

Procedure

The tests were carried out always at the same time of day, between 9.00 and 12.00 a.m.

Analgesia. Analgesia in DBA mice was measured by the hot plate method [3]. The time in seconds from the contact with the plate (55°C) until a hindpaw or forepaw lick, or jump occurred was recorded as response latency. Mice which failed to react within 30 sec were removed and a latency of 30 sec was recorded.

Activity. Spontaneous locomotor activity in C57 mice was measured in a rectangular corridor formed by an outer opaque plastic box (24×24×30 cm) containing an opaque plastic smaller one (14×14×30 cm). The number of corner crosses during a 3 min period after placing the naive animal in the corridor served as a measure of locomotor activity.

Treatment. The long-acting antagonists were applied subcutaneously 24–120 hr before morphine hydrochloride (10 mg/kg calculated as the base, IP). All tests were carried out 30 min after morphine injection. The efficacy of long-acting antagonists was assessed by comparing the data to baseline NaCl (0.9%) and morphine values, as well as to the antagonistic potency of the parent compounds naloxone and naltrexone. Furthermore the effects of long-acting antagonists alone on analgesia and activity were evaluated. For each drug dose and time interval different groups of mice (N=8–10) were used. To assess mortality for a given drug dose and time interval after injection (i.e., 24, 48, 72 hr) the number of animals in groups with longer testing intervals were included to those of shorter ones.

Drugs. The parent substances naloxone and naltrexone were provided by Salars, Como Italy.

β-Chlornaltrexamine (β-CNA), an alkylating nitrogen mustard derivative of naltrexone, was synthetized according to the literature [16].

β-Funaltrexamine (β-FNA), a fumaramate methyl ester derivative of naltrexone, was synthetized according to the literature [18], with the following exceptions: (a) the starting amine, obtained according to the literature [9], was separated from its isomer through silica gel column chromatography using ethylacetate-methanol-ammonia 30% (100:30:3) as eluent. (b) β-FNA base was purified through silica gel column chromatography and was eluated with diethylether-methanol (10:1). β-FNA was not converted to its hydrochloride. Solutions were made in 0.9% NaCl with 2 μl glacial acid for 1 mg drug.

Naloxazone (NXZ), a hydrazone derivative of naloxone, was synthetized according to the literature [15]. Solutions were made in 0.9% NaCl with 0.33 μl glacial acid for 1 mg drug.

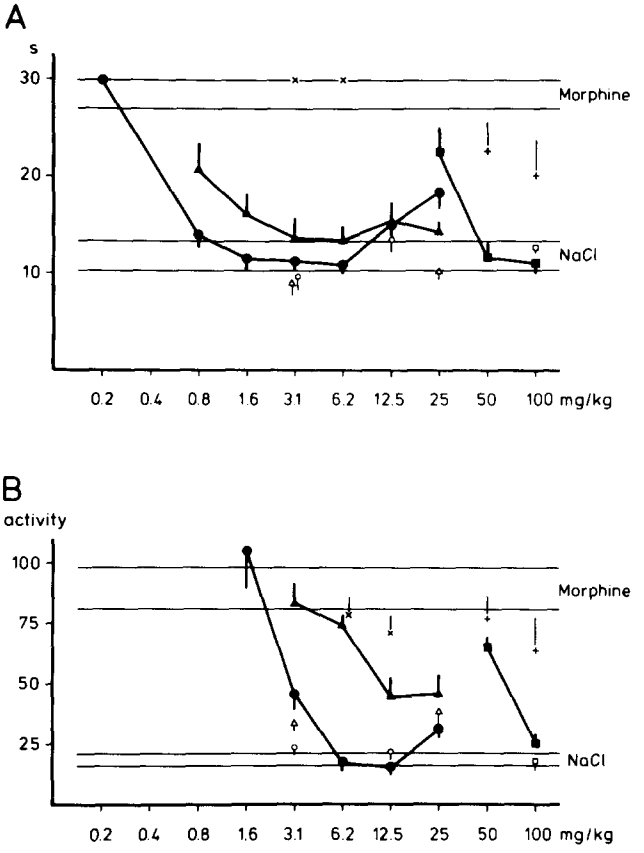

FIG. 1 A. Antagonism of morphine-induced analgesia (hot plate response latencies, mean±SE) in DBA mice 24 hr after injection of the antagonists β-CNA (●–●), β-FNA (▲–▲) and NXZ (■–■) as well as of naltrexone (x) and naloxone (+). Response latencies 24 hr after injection of antagonists alone are represented by open symbols: β-CNA (○), β-FNA (△), NXZ (□). The range (mean±SE) of baseline response latencies for NaCl or morphine injected animals is indicated by horizontal lines. B. Antagonism of morphine-induced locomotor activity (corner crosses, mean±SE) in C57 mice 24 hr after injection of the antagonists. For symbols see A.

All substances were injected at a volume of 0.1 ml/10 g body weight.

Statistics. The statistical significance of the results was ascertained by two-tailed Student's *t*-tests.

RESULTS

Antagonism of Morphine-Induced Analgesia in DBA Mice

The baseline response latencies for DBA mice in the hot plate test were for NaCl: 11.9±1.5 sec and for morphine: 28.6±1.4 sec. These ranges are represented in Fig. 1A by horizontal lines. Complete antagonism was defined as statistically not significant from the NaCl baseline, whereas lack of antagonism was defined as statistically not significant from the morphine baseline.

Antagonistic potency during 24 hr. Figure 1A shows that β-CNA, in doses of 0.8–12.5 mg/kg completely antagonized morphine-induced analgesia ($p>0.2$ vs. NaCl). Only partial antagonism was found with 25 mg/kg of β-CNA ($p<0.05$ vs. NaCl and morphine), a dose which at the same time resulted in a mortality rate of 22%. β-CNA alone (3.1 and 12.5 mg/kg)

TABLE 1

DURATION OF ANTAGONISTIC POTENCY OF DIFFERENT OPIATE ANTAGONISTS ON MORPHINE-INDUCED ANALGESIA IN DBA MICE (A) AND LOCOMOTOR ACTIVITY IN C57 MICE (B)*

		β-CNA		β-FNA		NXZ	
		0.8 mg/kg	3.1 mg/kg	1.6 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
A	24 hr	14.0 ± 1.2†	11.4 ±0.9†	16.2 ± 2.2†	14.3 ± 2.3†	11.7 ± 1.6†	11.3 ± 1.2†
	48 hr	14.3 ± 2.0†	8.9 ± 0.5†	25.9 ± 2.1§	13.2 ± 1.6†	22.7 ± 2.9§	18.9 ± 2.8‡
	72 hr	10.5 ± 0.8†	12.3 ± 2.7†	—	20.9 ± 2.8‡	—	19.2 ± 2.3‡
	120 hr	23.5 ± 2.0§	18.9 ± 3.0†	—	—	—	—

		β-CNA		NXZ
		6.2 mg/kg	12.5 mg/kg	100 mg/kg
B	24 hr	17.4 ± 2.9†	15.4 ± 3.6†	25.9 ± 3.8†
	48 hr	24.4 ± 3.6†	25.9 ± 6.7†	28.3 ± 6.4†
	72 hr	53.9 ± 7.2‡	23.7 ± 3.9†	40.3 ± 9.8†

*Data are expressed as mean hot plate latencies (mean ± SE) and mean corner crosses (mean ± SE), and were compared to NaCl and morphine baseline values.

†ns vs. NaCl and $p<0.01$ vs. morphine; ‡$p<0.05$ vs. NaCl and $p<0.05$ vs. morphine; §$p<0.01$ vs. NaCl and ns vs. morphine.

had no effect on hot plate latencies ($p>0.3$ vs. NaCl) and naltrexone (3.1 and 6.2 mg/kg) showed no long-term antagonistic potency ($p>0.3$ vs. morphine).

For β-FNA in doses of 1.6–25 mg/kg, a complete antagonism was found ($p>0.1$ vs. NaCl), while only a partial antagonism was evident at a low dose (0.8 mg/kg, $p<0.05$ vs. NaCl and morphine). Three percent of animals died after injection of 25 mg/kg. β-FNA alone (3.1 and 25 mg/kg) had no effect on hot plate latencies ($p>0.2$ vs. NaCl).

NXZ, in doses of 50 and 100 mg/kg completely antagonized morphine-induced analgesia ($p>0.7$ vs. NaCl). Only a tendency of an antagonistic effect was observed with 25 mg/kg ($p<0.1$ vs. morphine). 100 mg/kg of NXZ resulted in a mortality rate of 5% and the rate increased to 50% after 200 mg/kg. NXZ alone (100 mg/kg) had no effect on hot plate latencies ($p>0.5$ vs. NaCl). Fifty and 100 mg/kg of naloxone showed a slight and significant antagonistic effect ($p<0.1$ and <0.05 vs. morphine, respectively). Nevertheless, the antagonistic potency of NXZ was significantly higher compared to that of naloxone (50 mg/kg: $p<0.01$, 100 mg/kg: $p<0.05$).

Comparing the three antagonists, the minimal dosage which completely antagonized morphine-induced analgesia after 24 hr in DBA mice were 0.8, 1.6 and 50 mg/kg for β-CNA, β-FNA and NXZ, respectively. β-CNA and β-FNA proved to be much more potent than NXZ. β-CNA was significantly more effective than β-FNA at a dose of 0.8 mg/kg ($p<0.05$), whereas at higher doses this difference decreased (1.6 mg/kg: $p<0.1$, 3.1 mg/kg: $p>0.3$).

Antagonistic potency during 48–120 hr. β-CNA (0.8 mg/kg, the minimal effective dose for 24 hr) completely antagonized the morphine effect during 72 hr (see Table 1A). After 120 hr the antagonistic effect vanished. A higher dose of β-CNA (3.1 mg/kg) increased the duration of complete antagonism to 120 hr. It has to be mentioned that with longer intervals after injection the mortality rate increased, e.g., a dose of 12.5 mg/kg resulted in 0%, 29% and 42% mortality after 24, 48 and 72 hr, respectively.

In contrast, 1.6 mg/kg of β-FNA (the minimal effective dose for 24 hr) were ineffective after 48 hr. 25 mg/kg of β-FNA had to be applied in order to obtain a complete antagonism for 48 hr. After 72 hr this dose resulted still in a partial antagonism and there was no evidence for an increased mortality rate compared to 24 hr.

Similarly, 50 mg/kg of NXZ (the minimal effective dose for 24 hr) were ineffective after 48 hr. If the dose was increased to 100 mg/kg a partial antagonism was found during 72 hr. However, the mortality rate increased from 5% after 24 hr to 24% after 72 hr.

Comparing the three antagonists, only β-CNA showed a complete antagonism of morphine-induced analgesia during 72–120 hr in non toxic doses (0.8 and 3.1 mg/kg). Toxic doses of β-FNA (25 mg/kg) had to be used to completely block the morphine effect for 48 hr, or partially for 72 hr. Only toxic doses of NXZ (100 mg/kg) resulted in a partial antagonism for 48 and 72 hr.

Antagonism of Morphine-Induced Activity in C57 Mice

The baseline activity for C57 mice was for NaCl: 18.8±2.3 and for morphine: 89.8±8.7 corner crosses during 3 min. Similarly as for analgesia, antagonism was determined by comparing the data to NaCl and morphine baseline values.

Antagonistic potency during 24 hr. Figure 1B shows that β-CNA, in doses of 6.2 and 12.5 mg/kg completely antagonized morphine-induced activity ($p>0.4$ vs. NaCl). Only partial antagonism was found with 3.1 and 25 mg/kg ($p<0.01$ vs. NaCl and morphine). A high mortality rate (60%) was observed after injection of 50 mg/kg. β-CNA alone (3.1 and 12.5 mg/kg) had no effect on activity ($p>0.2$ vs. NaCl) and naltrexone (6.2 and 12.5 mg/kg) showed no long-term antagonistic potency ($p>0.1$ vs. morphine).

β-FNA (12.5 and 25 mg/kg) resulted in a significant antagonism ($p<0.002$ vs. morphine), but did not reach the NaCl baseline ($p<0.01$ vs. NaCl). This might have been due to the significant increase of activity 24 hr after injection of β-FNA alone (3.1 and 25 mg/kg: $p<0.01$ vs. NaCl). If the values of

β-FNA alone were considered as baseline, a complete morphine antagonism was evident for 12.5 and 25 mg/kg of β-FNA ($p>0.3$ vs. β-FNA alone). Increasing the dose from 25 to 50 mg/kg resulted in an increase of the mortality rate from 3% to 70%.

Only 100 mg/kg of NXZ resulted in a complete antagonism ($p>0.1$ vs. NaCl), while a partial blockade was evident after 50 mg/kg ($p<0.05$ vs. NaCl and morphine). The mortality rate for 100 mg/kg was 18% and increased to 40% after 200 mg/kg. NXZ alone (100 mg/kg) had no effect on activity ($p>0.8$ vs. NaCl) and naloxone (50 and 100 mg/kg) showed no long-term antagonistic effect ($p>0.1$ vs. morphine).

Comparing the three antagonists, the minimal dose which completely antagonized morphine-induced activity after 24 hr in C57 mice were 6.2, 12.5 and 100 mg/kg for β-CNA, β-FNA and NXZ, respectively. β-CNA and β-FNA proved to be more potent than NXZ, which had to be used in toxic doses. β-CNA was more potent than β-FNA, since with 3.1 and 6.2 mg/kg of β-CNA a significant antagonism was evident, whereas these doses of β-FNA were ineffective.

Antagonistic potency during 48–72 hr. β-CNA (6.2 mg/kg, the minimal effective dose for 24 hr) completely antagonized the morphine effect during 48 hr (see Table 1B). After 72 hr a partial antagonism was evident. A higher dose (12.5 mg/kg) increased the duration of complete antagonism to 72 hr. It should be mentioned that doses of 12.5 and 25 mg/kg which did not result in mortality after 24 hr showed mortality rates of 6% and 22% after 72 hr, respectively.

Due to the effect of β-FNA alone on activity, its prolonged antagonistic potency was not further investigated.

One hundred mg/kg of NXZ (the minimal effective dose for 24 hr) completely antagonized the morphine effect during 72 hr. However, the mortality rate increased from 18% after 24 hr to 40% after 72 hr.

Comparing β-CNA and NXZ, β-CNA proved to be more potent, since a non toxic dose (6.2 mg/kg) completely antagonized morphine-induced activity for 48 hr, and partially for 72 hr. Toxic doses had to be used for both substances (β-CNA: 12.5 mg/kg, NXZ: 100 mg/kg) to reach a complete antagonism during 72 hr.

DISCUSSION

Comparing the three compounds, β-CNA proved to be the most potent long-acting antagonist concerning morphine-induced analgesia in DBA mice as well as motor activity in C57 mice. The minimal doses which completely blocked morphine-induced analgesia and activity after 24 hr were for β-CNA: 0.8 and 6.2 mg/kg, for β-FNA: 1.6 and 12.5 mg/kg and for NXZ 50 and 100 mg/kg, respectively. The higher efficacy of all compounds in the DBA strain (factor 8 for β-CNA and β-FNA, factor 2 for NXZ) might have been due either to the mechanism underlying different morphine responses measured in the two strains, or to strain pecularities, e.g., in the uptake and metabolism of the antagonists or in the opiate receptor turnover. Two points do not favor a lowered uptake of opioids in the C57 strain: Firstly, brain levels of morphine, a compound which has a similar structure as the antagonists, were higher in C57 compared to DBA mice, 30 min after morphine injection or 24 hr after morphine pellet implantation [1]. Secondly, a low dose of β-FNA (3.1 mg/kg), blocking analgesia in DBA mice, had no antagonistic potency in C57 mice, but when given alone significantly increased locomotion in the C57 strain, suggesting an effective uptake. It is therefore quite likely that the lower potency of the antagonists in the C57 strain is due to the different composition and dynamics of the opiate receptor population [19]. It might be interesting to note that β-FNA given alone had an outstanding intrinsic activity in the C57 strain which makes interpretation concerning antagonistic potency and selectivity difficult.

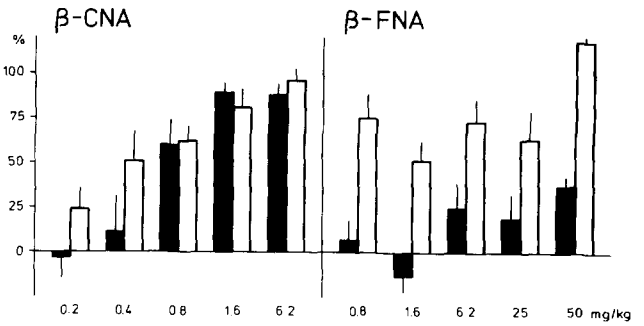

FIG. 2. Percent antagonism (mean±SE) of morphine-induced analgesia (■) and locomotor activity (□) in C3H mice 24 hr after injection of the antagonists β-CNA and β-FNA.

EXPERIMENT 2

Experiment 2 assessed the antagonistic potency of β-CNA and β-FNA in C3H/He mice. In this strain morphine induces a clear analgesic response as well as an increase in motor activity. This allows to detect an eventual specificity of the antagonists to block morphine-induced analgesia or locomotion.

METHOD

Subjects

Male mice of the C3H/He (C3H) strain, bred in our laboratory were used. They were housed (Macrolon cages 26×20×14 cm) in groups of three animals and tested at an age of 8–10 weeks (24–26 g).

Procedure

Each animal was tested for activity followed by the hot plate test, both according to Experiment 1.

β-CNA and β-FNA were injected (SC) 24 hr before morphine hydrochloride (20 mg/kg, calculated as the base, IP). All tests were carried out 30 min after morphine injection. In addition, the effects of β-CNA and β-FNA alone were tested. For each drug dose different groups of mice (N=8–10) were used.

For each animal the percentages of antagonism of morphine-induced analgesia and activity were calculated, with the difference between NaCl and morphine baselines being 100%.

RESULTS AND DICUSSION

The baseline values for C3H mice in the hot plate test were for NaCl: 6.6±0.5 sec, for morphine: 24.6±3.1 sec, those of the activity test were for NaCl: 17.4±3.9 corner crosses, for morphine: 77.1±8.4 corner crosses.

β-CNA dose dependently antagonized both morphine-induced analgesia as well as morphine-induced activity (see Fig. 2). There was no significant difference in the percentage of morphine antagonism between both responses for doses of

0.2–6.2 mg/kg ($p>0.1$). In contrast, β-FNA was much less potent in antagonizing morphine-induced analgesia in comparison to the activity response. Only a tendency to block morphine-induced analgesia was evident with a high dose of 50 mg/kg ($p<0.1$ vs. morphine), while β-FNA inhibited morphine-induced activity at doses from 0.8–50 mg/kg ($p<0.02$ vs. morphine). The difference in the percentage of morphine antagonism between both responses was significant ($p<0.001$ to $p<0.05$) at all doses of β-FNA tested. β-FNA (1.6, 6.2 and 25 mg/kg) and β-CNA (1.6 and 6.2 mg/kg) given alone had no effect on hot plate latencies and activity measure, when tested 24 hr after the injection ($p>0.1$ vs. NaCl).

The results suggest a selective antagonistic potency for β-FNA, while β-CNA indiscriminately blocks both morphine responses.

GENERAL DISCUSSION

In two different in vivo measurements of morphine action, i.e., analgesia and increase in activity, carried out in three different inbred strains of mice, β-CNA turned out to be the most effective long-term antagonist among the three compounds tested, while β-FNA was the most selective compound. In non toxic dosages, β-CNA blocked morphine-induced analgesia in DBA mice up to 120 hr. This is in accordance with previous findings showing an inhibitory effect of intracerebroventricularly injected β-CNA on morphine-induced analgesia for up to 72 hr [17]. Higher dosages of β-FNA had to be used in the DBA and C57 strains to block morphine effects and the duration of antagonism was shorter in comparison to β-CNA, as demonstrated in the DBA strain. Similarly, 2 hr after intracerebroventricular administration of β-CNA the increase of the morphine ED_{50} was 13 times higher than after application of equal doses of β-FNA [16,24]. These findings parallel in vitro studies where lower concentrations of β-CNA were necessary to antagonize morphine effects on the mouse vas deferens and guinea pig ileum preparations [22,25].

Regarding selectivity it was reported that β-FNA preferentially antagonizes μ-receptor mediated responses [22, 24, 25]. The low antagonistic potency of β-FNA in C57 mice (Experiment 1), where morphine induces a suggested δ-response, would support such a selectivity. In mice of the C3H strain (Experiment 2) β-FNA proved to be a selective antagonist in contrast to β-CNA. However, the selectivity of β-FNA was not observed in the expected μ-response (analgesia), in that the compound more selectively antagonized morphine-induced locomotion (an assumed δ-response). This rather unexpected finding suggests that strain differences have to be considered when selectivity for a specific morphine response is assessed. It seems that in a strain like C3H where morphine induces a mixed analgesic/activity response, the concept of associating a certain morphine response to a specific receptor type is too trivial. How can one explain the fact that a selective μ-antagonist like β-FNA does not more effectively antagonize the presumed μ-response in C3H mice? In the case of μ- as well as κ-receptor mediated analgesia, the high antagonistic selectivity of β-FNA to μ-receptors [22, 24, 25] would still make feasible a morphine-induced analgesia via κ-binding sites. However, this does not explain why the μ-antagonist β-FNA effectively blocks the morphine-induced activity response. The results suggest that the activity response induced by morphine is mediated by different opiate receptor subtypes in the C3H and C57 strains. Speculatively, the locomotor activity response in the C3H strain might be due to δ-receptor as well as μ-receptor activation, or blockade of the μ-receptor might inhibit binding of the agonist to the δ-receptor. Similarly it was hypothesized that blockade of the δ-receptor inhibits coupling of morphine to the μ-receptor, attenuating analgesia [23].

In our experiments naloxazone failed to be a suitable long-term opiate antagonist for in vivo studies, since in most cases toxic doses had to be used. Only morphine-induced analgesia in DBA mice was blocked in a non toxic dose during 24 hr. Higher toxic doses antagonized morphine-induced activity (C57 mice) and analgesia for longer than 24 hr, which is in agreement with previous work [14]. It was recently put forward that the low efficacy is due to the fact that naloxazone is not the active principle, but that in acidic solution it rearranges spontaneously to naloxonazine [7,8]. Naloxonazine blocked opiate binding in vitro 40 times more potently than naloxazone, and no long-term effect was found when the azine formation was prevented [7]. Interestingly, we have found no long-term antagonistic potency of dimethylnaloxazone (in doses up to 100 mg/kg), a compound which cannot form an azine (unpublished results).

In conclusion, inbred strains of mice served as a useful tool for the evaluation of long-acting opiate antagonists on different in vivo morphine actions. Whatever morphine response tested in the three strains, β-CNA turned out to be the most reactive compound, while β-FNA showed a strain dependent antagonistic potency and selectivity. The results do not support a one receptor one response hypothesis of opiate mechanisms and caution against extrapolation from in vitro assessed receptor selectivity to an antagonism of a specific morphine-induced response. Both naltrexone derivatives might be of importance for the investigation of the physiological and behavioral consequences of endogenous opiate blockade.

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